# Adjuvants in HIV Vaccine Research

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New vaccines are presently under development and testing for the control of infectious diseases including HIV. Several of these vaccines are composed of synthetic, recombinant, or highly purified subunit antigens. Subunit vaccines are designed to include only the antigens required for protective immunization and to be safer than whole-inactivated or live-attenuated vaccines. However, the purity of the subunit antigens and the absence of self-adjuvanting immunomodulatory components associated with attenuated or killed vaccines often results in weaker immunogenicity of subunit vaccines. Immunologic adjuvants are agents that enhance specific immune responses to vaccines. Formulation of vaccines with potent adjuvants is an attractive approach for improving the performance of vaccines composed of subunit antigens. Adjuvants have diverse mechanisms of action and should be selected for use based on the route of administration and the type of immune response (antibody, cell-mediated, or mucosal immunity) that is desired for a particular vaccine. Adjuvant mechanisms of action include: 1) increasing the biological or immunological half-life of vaccine antigens; 2) improving antigen delivery to antigen presenting cells (APC) and antigen processing and presentation by the APC; and 3) inducing the production of immunomodulatory cytokines. Through modulation of cytokine responses, adjuvant formulations can be designed that favor the development of Th1 (type 1) or Th2 (type 2) immune responses to vaccine antigens. Novel adjuvants are presently undergoing preclinical and clinical testing with experimental human candidate vaccines. Standardized preclinical adjuvant safety tests to support the clinical evaluation of novel adjuvants are also under development.

## Introduction

An immunologic adjuvant may be defined as any substance that when incorporated into a vaccine formulation acts generally to accelerate, prolong, or enhance the quality of specific immune responses to vaccine antigens. The word adjuvant is derived from the Latin word *adjuvare*, which means to help or aid. Immunologic adjuvants have been under development and testing for most of this century. Ramon, in the mid-1920s, observed that horses that developed abscesses at the site of an injection of diphtheria toxoid produced higher antitoxin titers than animals without abscesses. He later reported that abscesses induced by the injection of foreign substances together with toxoid also augmented anti-toxin responses in horses [1,2]. In 1926, Glenny demonstrated the adjuvant activity of aluminum compounds using an alum-precipitated diphtheria toxoid vaccine [3]. In the mid-1930s Jules Freund developed a powerful immunologic adjuvant composed of a water-in-mineral oil emulsion and containing killed mycobacteria as an additional immunomodulator [4]. This adjuvant is known as Freund's complete adjuvant (FCA). Although FCA is one of the most effective adjuvants known, is highly reactogenic and cannot be used in human vaccines. However, Freund's incomplete adjuvant (FIA), which does not contain mycobacteria was employed in an influenza vaccine licensed in the United Kingdom and is used in several HIV vaccines under clinical evaluation. In 1956 Arthur Johnston discovered the adjuvant activity of endotoxins from Gram-negative bacteria [5] and in 1974 Ellouz and colleagues identified muramyl dipeptide (MDP) as the smallest adjuvant-active component of the mycobacteria in FCA [6]. Presently, aluminum salt-based adjuvants continue to be the only immunologic adjuvants used in U.S.-licensed vaccines. However, hundreds of natural and

synthetic compounds have been identified that posses adjuvant activity. A variety of these novel adjuvants, which may be used to augment or replace alum in human vaccines, have been under development and in preclinical evaluation for several decades [7]. In animal models, many novel adjuvants have been demonstrated to be more effective than alum in enhancing both antibody and cell-mediated immune responses to vaccine antigens. Extensive preclinical evaluation of novel immunologic adjuvants have been conducted and clinical trials comparing the activities of various adjuvants have been initiated.

**Advantages of the Use of Adjuvants**

Potential advantages of the use of immunologic adjuvants in vaccine formulations include there ability to: 1) direct and optimize immune responses that are appropriate for the vaccine; 2) enable mucosal delivery of vaccines; 3) promote cell-mediated immune responses; 4) enhance the immunogenicity of weaker immunogens such as highly purified or recombinant antigens; 5) reduce the amount of antigen or the frequency of immunization required to provide protective immunity; 6) improve the efficacy of vaccines in individuals with reduced or weakened immune responses such as newborns, the aged, and immunocompromised vaccinees.

**Types of Immunologic Adjuvants**

Immunologic adjuvants can be classified by their sources, mechanisms of action, and physical or chemical properties. Table 1 lists examples of the types of adjuvants under development and testing for use with human vaccines.

**Adjuvant Mechanisms of Action**

Adjuvants have diverse mechanisms of action and must be chosen for use with a particular vaccine based on the route of administration to be employed and the type of immune responses desired.

The first mechanism of adjuvant action identified was the so-called depot effect, in which gel-type adjuvants such as aluminum hydroxide or emulsion-based adjuvants such as IFA associate with antigen and facilitate transport of antigen to the draining lymph node where immune responses are generated. Immunogenicity of small antigens such as synthetic peptides that otherwise would be rapidly cleared from the injection site and draining lymph nodes can be improved by the use of adjuvants that form particles or otherwise associate with and hold antigen. Adjuvants can also act through enhancement of antigen presentation. Immunologic adjuvants act directly or indirectly on antigen presenting cells (APC) such as macrophages and dendritic cells [8,9]. The emulsion-based adjuvant MF59 has recently been shown to be internalized by dendritic cells [10]. Certain novel adjuvants such as purified saponins, ISCOMS, and liposomes have been shown to greatly improve the induction of MHC class-I-restricted CD8+ CTL responses over those induced by the same antigen given alone or in combination with standard alum adjuvants [11-13]. A mechanism by which these adjuvants induce CTL may be through the delivery of antigen directly to the cytosol for presentation with MHC class I molecules [9]. Cytosolic antigen delivery by membrane-active adjuvants could mimic antigen presentation that occurs during viral infection or immunization with live-attenuated vaccines. Antigen presented to the cytosol could bypass endosomal antigen delivery and subsequent processing with MHC class II molecules, which occurs when antigen is delivered alone or in alum and induces primarily antibody responses [14] via presentation to CD4+ T-helper lymphocytes. Adjuvants may also promote cytosolic antigen delivery and MHC class I presentation by enabling antigen to cross endosomal membranes into the cytosol after ingestion of antigen-adjuvant complexes by APC [15]. Antigen can be targeted

to macrophages or dendritic cells by particulate adjuvants such as liposomes, and APCs can be stimulated by adjuvants to secrete immunomodulatory cytokines. Various cytokines induced by adjuvants act on lymphocytes to promote predominately Th1 or Th2 immune responses [14,16,17]. Adjuvants that enhance Th1 immune responses through the induction of IFN-γ and delayed-type hypersensitivity (DTH) also elicit the production of IgG subclasses that fix complement and bind with high affinity binding to FcγI receptors (e.g., IgG2a in mice and IgG1 in humans) [18-20]. These immunoglobulin subclasses are the most active in complement-mediated lysis and antibody-dependent cell-mediated cytotoxicity (ADCC) effector mechanisms. Several cytokines are under evaluation as vaccine adjuvants, including IL-2, interferon gamma (IFN-γ, GM-CSF, and IL-12 [21-23]. IL-12 is a recently characterized cytokine that may play a pivotal role in immunomodulation by various immunologic adjuvants [24-26]. Jankovic *et al*. showed that the addition of IL-12 to an alum-adsorbed HIV-1 gp120 vaccine elicited type 1 (Th1) cytokines and IgG2 and IgG3 antibody responses in mice. The same vaccine without IL-12 induced type 2 (Th2) cytokines and IgG1 antibody responses [27]. Adjuvant-active bacterial toxins such as cholera toxin, and pertussis toxin, which preferentially drive Th2-like responses, have been shown to enhance IgA and IgE [17,28-30] antibody production. Adjuvants that drive Th2-like immune responses could enhance protection against mucosal virus transmission through augmentation of IgA production.

**Adjuvant Safety**

The benefits of incorporating adjuvants into vaccine formulations to enhance immunogenicity must be weighed against the risk of these agents to induce adverse reactions. Local adverse reactions include local inflammation at the injection site or, rarely, the induction of granulomas or

sterile abscesses formation. Systemic reactions to adjuvants observed in laboratory animals include malaise, fever, adjuvant arthritis, and anterior uveitis [31,32]. Such reactions often are caused by the interaction of the adjuvant with the antigen itself, or may be due to the type of response or cytokine profile the adjuvant produces to a particular antigen. Therefore, even though separate and extensive preclinical toxicology and safety studies have been performed on both the adjuvant and the vaccine antigens, a final safety evaluation of the human candidate vaccine formulation proposed for Phase I clinical testing should be conducted. This evaluation should be conducted in a small animal species in which the antigen has been found to be immunogenic and that can be reproducibly immunized via the same route proposed for the human clinical trials. The dose and frequency of immunization of the vaccine also should meet or exceed those anticipated for use in the clinical trial. Such a test, recently designed by a collaborative effort between Center for Biologics Evaluation and Research, Food and Drug Administration (CBER/FDA) and the National Institute of Allergy and Infectious Diseases (NIAID) [33], has been used to evaluate with several vaccine formulations containing novel adjuvants (Figure 1).

**Future Directions**

Adjuvant research is a rapidly advancing field reflecting the rates at which new adjuvants are discovered and the better understanding of immune mechanisms possible because of advances in immunobiology. In turn, adjuvants should now be applied to the study of many aspects of basic immunology. For example, adjuvants can be used as a tool to study immune mechanisms such as antigen presentation by dendritic cells (DC) and modulation of immune responses by cytokines and their receptors. Adjuvants can also be employed in vaccine design research which could assist

in identifying the requirements of protective immunity, since different adjuvants vary immune responses to the same experimental antigen. The activities of adjuvants in humans as compared with their effect in small animals should be more fully evaluated. Animal models should be developed that can predict as accurately as possible the effectiveness in humans of a particular adjuvant when formulated with the desired vaccine antigens.

**Summary**

Development of safe and effective vaccines composed of subunit antigens will require the ability to selectively drive appropriate protective immune responses to them. The use of immunologic adjuvants to enhance and direct immune responses to subunit vaccines is a critical component of a rational vaccine design. Adjuvants have diverse mechanisms of action and must be selected for use based on the immune responses (e.g., antibody, mucosal, CTL) contributing to the induction of protective immunity. Adjuvants can improve the performance of vaccines by targeting of antigen to APC, eliciting cytokines that direct Th1 or Th2 immune responses, promoting cell-mediated immunity including CTL, and reducing the number of immunizations or the amount of antigen required for protective immunization. The selection of a vaccine adjuvant should be based on analysis of the potential benefit of the adjuvant in enhancing the immunogenicity of a vaccine weighed against its risk to induce adverse local or systemic reactions. The severity and prevalence of the disease against which the vaccine is designed to afford protection may also be considered in risk and benefit determinations for the use of novel adjuvants. Standardized methods to evaluate adjuvant safety should be implemented for human vaccines that are to be formulated with novel adjuvants.

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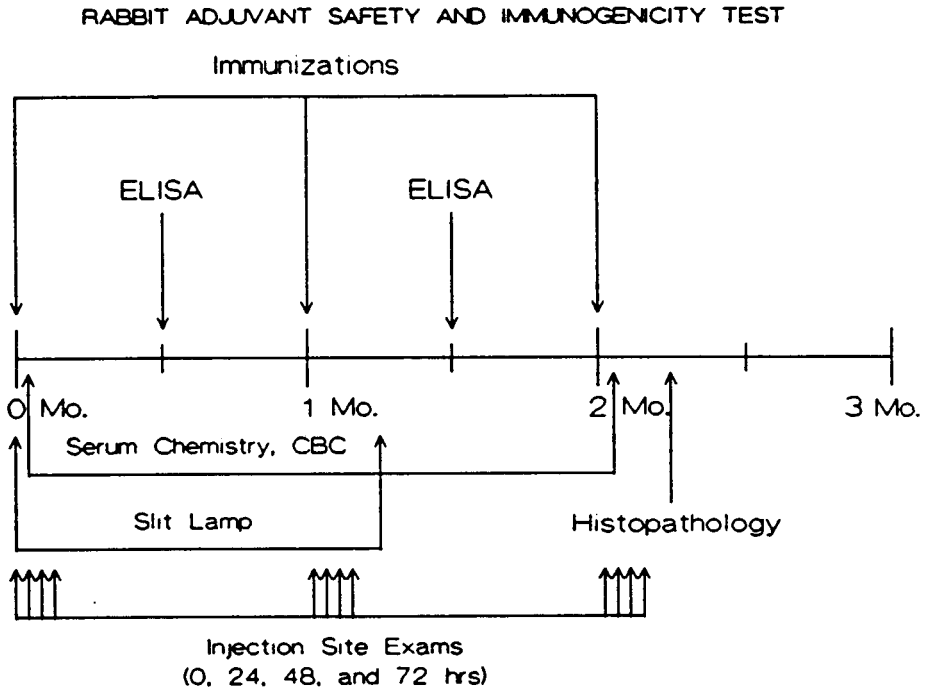
*Table I.* Types of Immunologic Adjuvants

| | | |
|---|---|---|
| Gel-type adjuvants | Aluminum hydroxide/aluminum phosphate) [3,34] | |
| | Calcium phosphate [34] | |
| Microbial adjuvants | DNA | CpG motifs [35] |
| | Endotoxin | Monophosphoryl lipid A [36] |
| | Exotoxins | Cholera toxin [28,37] |
| | | *E. coli* heat-labile toxin [38-40] |
| | | Pertussis toxin [41,42] |
| | Muramyl dipeptide | (MDP) [6,43] |
| Oil-emulsion and Emulsifier-based adjuvants | Freund's Incomplete Adjuvant (IFA) [44,45] | |
| | MF59 [10,46,47] | |
| | SAF [18,48,49] | |
| Particulate adjuvants | Immunostimulatory complexes (ISCOMs) [45,48,50] | |
| | Liposomes [51,52] | |
| | Biodegradable microspheres [53,54] | |
| | Saponins (QS-21) [12,55] | |
| Synthetic adjuvants | Nonionic block copolymers [56,57] | |
| | Muramyl peptide analogues [43,58,59] | |
| | Polyphosphazene [60,61] | |
| | Synthetic polynucleotides [62,63] | |

**Figure Legend**

*Fig. I.* Rabbit adjuvant safety and immunogenicity test. Six to 10 rabbits per group. Route of immunization is the same route that is proposed for clinical trials. Rabbits receive the highest dose of vaccine per injection that is proposed for Phase 1 clinical trials. Rabbits should receive one additional injection over the number to be administered to humans. Serum chemistry panel should include creatine phosphokinase (CPK). Complete blood count (CBC).

Figure I


RABBIT ADJUVANT SAFETY AND IMMUNOGENICITY TEST
Immunizations
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Slit Lamp
Histopathology
Injection Site Exams
(0, 24, 48, and 72 hrs)